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A Study of the Relationship Between Certain Internal and External Morphological Changes Occurring During Induced and Natural Metamorphosis in *Rana pipiens* and *Rana catesbeiana*

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(Text-figures 1-12)

THE dramatic metamorphosis of the tailless Amphibia is the subject of an extensive literature resulting in large part from Gudernatsch's (1912, 1914) acceleration of the process by thyroid feeding, and from the subsequent discovery of the thyroid-pituitary relationship by Adler (1914), Allen (1916) and Smith (1916a). A chronology of the external metamorphic events was established by Etkin (1932), who also showed (1935) that thyroxine treatment did not change the order of events but did change their spacing. Knowledge of internal metamorphosis both normal and accelerated is quite complete, with many excellent and detailed studies of one, two, or more internal organs. However, there is no single analysis of internal changes comparable to Etkin's for the external pattern. Disharmonies in development during accelerated metamorphosis have frequently been noted, and several theories of the control of the sequence and spacing of metamorphic events have been reported (see the review of Lynn & Wachowski, 1951).

During normal metamorphosis, the rate and extent of change in the several organs are so correlated that a given degree of change in an external organ, such as the developing hind limb, is a measure of the change to be expected internally (Allen, 1929). Although the available evidence indicates that this is not true during accelerated metamorphosis, more information as to the degree of deviation from the normal correlation between internal and external changes in accelerated metamorphosis seemed desirable. Therefore, a comparison of the development of several internal organs in normal and thyroxine-treated animals at similar external metamorphic stages was undertaken. *Rana pipiens* and *Rana catesbeiana* were both studied in order to obtain differing lengths of larval period for the purpose of comparison.

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MATERIALS AND METHODS

1. *Plan of the Study*

Rana pipiens and *Rana catesbeiana* were the species chosen for study because of the difference in the lengths of their larval periods.

To compare the integration of internal and external changes in normal metamorphosis with that in accelerated metamorphosis, larval stages were selected at which the external morphology of the normal and treated animals was considered comparable. Normal and accelerated animals were killed at each of these stages and certain of their internal organs compared. Because of the occasional rather substantial variation in age between normal and accelerated tadpoles at apparently the same external metamorphic stage, untreated tadpoles of the same age as the accelerated were also killed and studied concurrently.

To select accelerated stages most comparable morphologically to normal stages, the effects of the various thyroxine concentrations were observed. It was concluded that only the forelimb emergence stage was recognizably similar to the

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normal in all series, and therefore a more comprehensive study was made of this stage. Earlier accelerated stages in certain respects overlapped several normal stages. Although accelerated animals were studied at a stage later than forelimb emergence, this later stage has been named "terminal stage" rather than "tail stub" stage as in the normal series.

The following stages were studied:

(1). Initial stage (normal animals, 12 and 21 days old)

(2). Two mm. hindlimb stage (*R. pipiens* only)

(3). Forelimb emergence stage (both forelimbs emerged)

(4). Tail stub (terminal stage)

The effects of rate of acceleration on metamorphic integration were studied by the use of two aqueous thyroxine concentrations: 1:10,000,000 and 1:100,000,000. Each has been used previously (e.g., Allen, 1932; Etkin, 1935). In addition, two age groups of tadpoles were treated with each concentration. One group was 12 days old at the beginning of treatment; the other 21 days old. Treatment by immersion was continuous.

The following groups for each stage in both species were studied and compared:

(1). Animals metamorphosing normally in the laboratory;

(2). Animals accelerated by 1:10,000,000 thyroxine from 12 days of age;

(3). Untreated animals of the same age as were those in (2) when killed for examination;

(4). Animals accelerated by 1:10,000,000 thyroxine from 21 days of age;

(5). Untreated animals of the same age as were those in (4) when killed for examination;

(6). Animals accelerated by 1:100,000,000 thyroxine from 12 days of age;

(7). Untreated animals of the same age as were those taken in (6) when killed for examination;

(8). Animals treated with 1:100,000,000 thyroxine from 21 days of age;

(9). Untreated animals of the same age as were those in (8) when killed for examination.

At the selected stages, internal metamorphosis was studied morphologically following dissection, and the forelimb stage of *R. pipiens* by microscopic examination of serial section. The internal organs studied were:

(1). *Tongue*: length, development, presence or absence or premetamorphic papillae;

(2). *Intestine*: length, histology (in *R. pipiens*) at the forelimb stage;

(3). *Gall bladder*: color, size;

(4). *Urinary bladder*: presence, degree of development;

(5). *Pancreas*: size, histology (in *R. pipiens*, forelimb stage);

(6). *Pituitary*: (in *R. pipiens*, forelimb stage) size, development;

(7). *Thyroid*: (in *R. pipiens*, forelimb stage) follicle number, mitotic activity, position.

In addition, body length, tail length and hindlimb length were recorded throughout the course of normal and accelerated metamorphosis in both species.

Because of *R. catesbeiana*'s long tadpole period, 17 late tadpoles were collected from the Kenilworth Lily Ponds, Washington, D. C., March 24, and brought to the laboratory to serve as the normal series for the stages of forelimb emergence and tail resorption. The rest of the *R. catesbeiana* tadpoles used for the study of younger normal stages and for the experimental series were laboratory raised from an egg film collected at the same place, July 2.

2. *Method of Rearing and Feeding the Tadpoles*

The tadpoles were reared in flat, white enameled pans (10" × 15" × 1½"). For *R. pipiens*, 20 animals were placed in each pan with 1,000 cc. of water or thyroxine solution. For *R. catesbeiana*, 10 animals were placed in each pan with 2,000 cc. of water or thyroxine solution. The thyroxine solutions and water were changed daily. Food (chopped spinach and Pablum) was provided in excess. The experiments terminated after forelimb emergence when the animals' tails were resorbed to dark stubs or when deaths had reduced the number of animals in each series to 10 for *R. pipiens* and to 5 for *R. catesbeiana*.

3. *The Normal and Experimental Series*

The experimental animals in each species were divided into four series. In the table below are listed the designation, the treatment and the number of animals in each series.

Naturally metamorphosing animals constituted the series designated as "SC" (Stage Controls). Separate series of untreated tadpoles were raised and observed, and individuals from these series were fixed concurrently with those from the experimental series. These constituted the "Age Controls." For *R. pipiens* there was a total of 100 tadpoles in the Age and Stage Control series; for *R. catesbeiana,* a total of 96 tadpoles.

Designation	Treatment	Number of animals *R. pipiens*	Number of animals *R. catesbeiana*
A-1	1:10,000,000 thyroxine from age 12 days	60	30
A-2	1:10,000,000 thyroxine from age 21 days	60	30
B-1	1:100,000,000 thyroxine from age 12 days	60	30
B-2	1:100,000,000 thyroxine from age 21 days	60	30

4. *External Measurements*

Measurements were made as often as changes in rate of development seemed to require. In both species, measurements were made daily during the course of the experiments using 1:10,000,000 thyroxine. For the 1:100,000,000 thyroxine series, generally daily measurements were made for *R. pipiens* and less frequently for *R. catesbeiana* (once a week or ten days). At such times, 10 animals in each series were measured. To facilitate the process of measuring, the normal and experimental animals of both species were anesthetized with MS 222.

5. *Dissection*

Two specimens of *R. pipiens* and three specimens of *R. catesbeiana* for each normal stage and each experimental stage, along with an equal number of age controls, were dissected. Gall bladder color changes and presence or absence of the urinary bladder were investigated in additional dissections made so that these observations were based on at least five dissections for each stage and series in both species. Dissection was done using 7× and 17× magnifications. The entire digestive tract was removed from the body cavity by freeing the cloaca from the ureters, cutting the mesentery and lifting the entire mass from the dorsal body wall anteriorly at the glottal level of the pharynx. Then the color of the gall bladder and its size relative to the liver were recorded. The shape of the ventral pancreas was sketched and its dimensions taken with dividers recorded on the sketch. The liver and pancreas were removed, the bile duct severed and the intestinal mesentery cut so that the entire intestine could be uncoiled. Measurement was accomplished by cutting the intestine into convenient lengths and straightening these lengths on a millimeter rule. Finally the presence or absence of the urinary bladder and its relative degree of development were recorded.

The shape of the tongue was sketched and (for *R. catesbeiana*) the anterior-posterior dimension as taken with dividers was recorded. The number and disposition of the premetamorphic papillae were also recorded.

6. *Histological Study*

Two animals in the forelimb emergence stage in each of the *R. pipiens* series were serially sectioned and prepared for histological study. Age controls were also serially sectioned. Specimens were fixed in Bouin's solution, sectioned in paraffin at 10 micra and stained with Masson's stain.

Observations

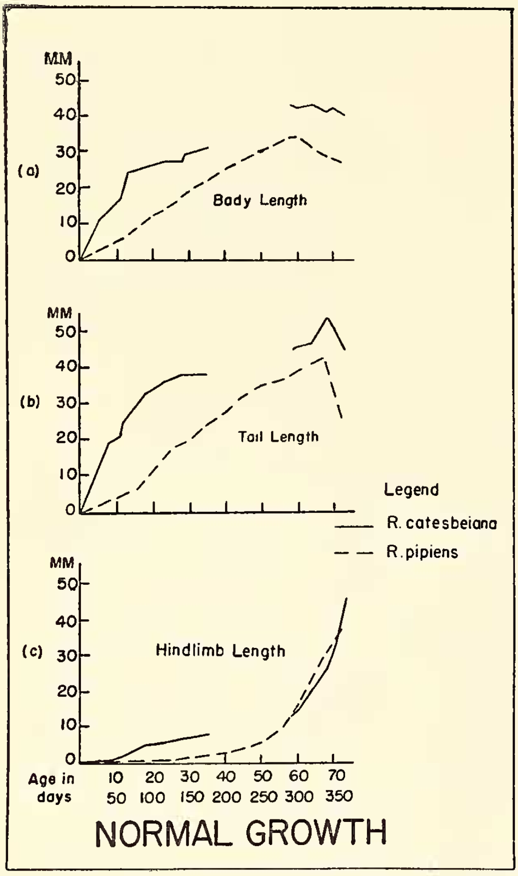
1. *Growth Measurements*

It has been noted already that the normal series of *R. catesbeiana* represents animals obtained at different times; (first) mature tadpoles collected in the field and raised in the laboratory through the rest of the tadpole period; and (second) tadpoles hatched in the laboratory from an egg film collected later in the season. The tadpoles from the egg film provided both the experimental series and their normal Age Controls. The Age Controls were observed during a period of 173 days and were fixed at the end of that time with the remaining experimentals. By this time, a number of the Age Controls were as large and as well developed as the late tadpoles (used as the Stage Controls) had been when collected. Therefore the growth data for the two normal groups were plotted as a single curve (Text-fig. 1) broken by a time lapse equal to that between December (when the Age Controls were fixed) and March (when the Stage Controls were collected).

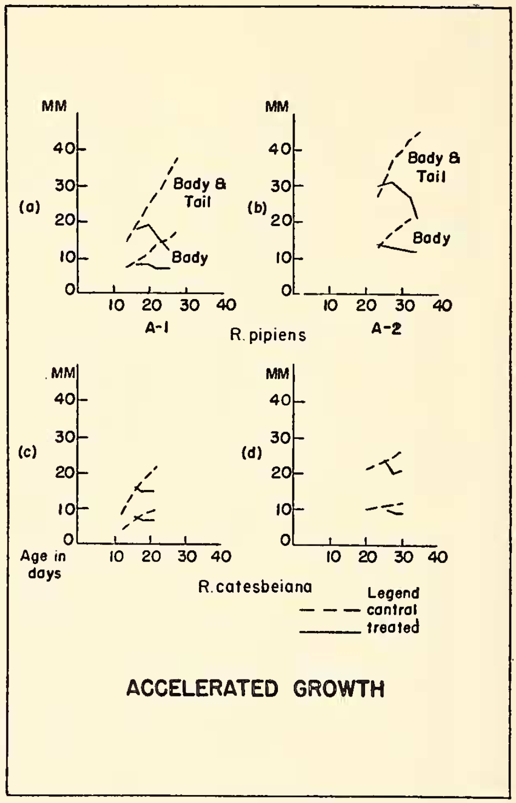
The growth curves and the time required for response (Text-fig. 2) were approximately the same in both species of organisms treated with 1:10,000,000 thyroxine. The experiments terminated in death after 16 days for *R. pipiens* and 10 or 11 days for *R. catesbeiana.* With 1:100,000,000 thyroxine, the growth curves for the two species are very similar (Text-figs. 3 & 4). The number of days required for response in *R. catesbeiana* was greater than for *R. pipiens,* but, considering the normal length of the respective tadpole periods, the effect of treatment appeared earlier in *R. catesbeiana* (see Text-fig. 5). It should be pointed out, however, that tail resorption was carried further in *R. pipiens* (Text-fig. 6) during this time.

The age difference in the groups of tadpoles treated with the same thyroxine concentrations caused no appreciable difference in the growth curves in either species.

The curves obtained for hindlimb growth of tadpoles treated with 1:100,000,000 thyroxine (Text-figs. 3 & 4) are almost identical with that published by Etkin (1935) for thyroidectomized *R. cantabrigensis* tadpoles. Relative to body length, the hindlimbs of the 1:100,000,000 thyroxine-treated animals were longer than normal in both species (Text-fig. 5).



TEXT-FIG. 1. Normal growth curves for (a) body length, (b) tail length, and (c) hindlimb length in *R. pipiens* and *R. catesbeiana.* One unit of age for *R. pipiens* has been made equal to five units for *R. catesbeiana* for easier comparison of the curves.



TEXT-FIG. 2. Growth under treatment with 1:10,000,000 thyroxine. A-1 curves are for tadpoles treated from 12 days of age; A-2 are for tadpoles treated from 21 days of age; (a) and (b) show data from treated *R. pipiens* tadpoles; (c) and (d) from *R. catesbeiana.*

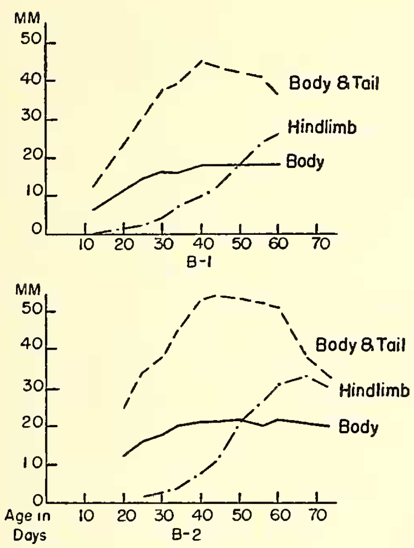
Continuous tail growth of both species of animals treated with 1:100,000,000 thyroxine (Text-fig. 6) was unexpected in view of Blacher's (1928) observation that short exposure to dilute concentrations of thyroidin produced tail shortening.

2. *Dissection Results*

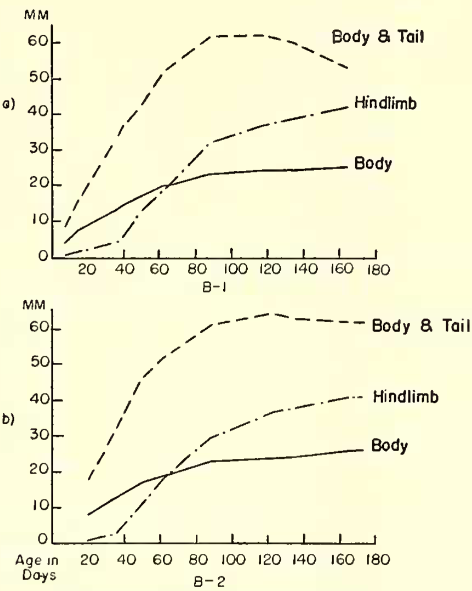
a. *Intestine*

Kuntz (1922) found that the average reduction in intestine length during the normal metamorphosis of *R. pipiens* was 82.2%. In the present investigation the average reduction in the intestine length of control animals was found to be 86.2% for *R. pipiens* and 83.7% for *R. catesbeiana.* These averages are in very good agreement if it is remembered that dietary differences may affect intestine length by as much as 58% (Babak, 1905).

Text-fig. 7a shows the changes in the ratio of intestine length to body length for the normal and for the accelerated *R. pipiens* series; in Text-fig. 7b, comparable data are presented for *R. catesbeiana.* It can be seen that, during normal metamorphosis, the ratio for the tadpole stage is 13 to 14 and it decreases to a value of approximately 2 at the tail resorption stage. By the time of forelimb emergence, one-half of the total shortening had occurred in the intestine of *R. catesbeiana* and three-quarters of the total shortening of the intestine of *R. pipiens* had been effected. Treatment with 1:10,000,000 thyroxine produced shortening of the intestine, giving a ratio (intestine length to body length) of 2; however, shortening had been completed by the forelimb emergence stage. Under the influence of 1:100,000,000 thyroxine, little shortening had taken place by forelimb emergence. By the terminal stage, the intestine length-body length ratio in *R. pipiens* is almost normal as compared with the ratio for *R. catebeiana* in which the intestine remains considerably longer than twice the body length.



TEXT-FIG. 3. *R. pipiens,* growth of tadpoles under 1:100,000,000 thyroxine treatment. B-1 curves are for tadpoles treated from 12 days of age; B-2 for tadpoles treated from 21 days of age.



TEXT-FIG. 4. Growth of *R. catesbeiana* tadpoles under 1:100,000,000 thyroxine treatment. (a) B-1 curves are for tadpoles treated from 12 days of age; (b) B-2 curves for tadpoles treated from 21 days of age.

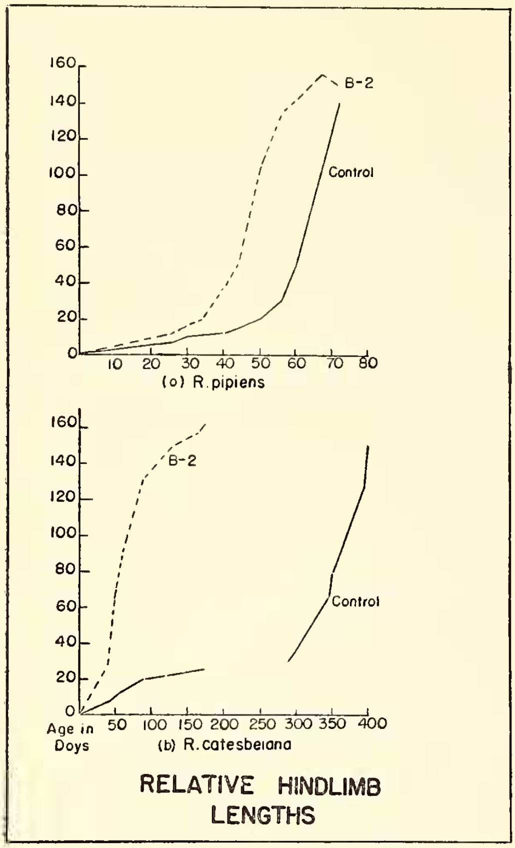
Blacher (1928) and Allen (1932) established the order of sensitivity to thyroid treatment of the several tadpole organs. Both Blacher and Allen reported the intestine to be one of the more sensitive organs by virtue of its shortening after even brief periods of exposure to dilute concentrations. In Text-fig. 7, the curves show that the intestine of tadpoles treated with 1:100,000,000 thyroxine continues to grow for some time prior too the onset of shortening. The data can be reconciled if, instead of shortening, one postulates a period of slower intestinal growth in the treated tadpoles than in controls of the same age.

b. *Pancreas*

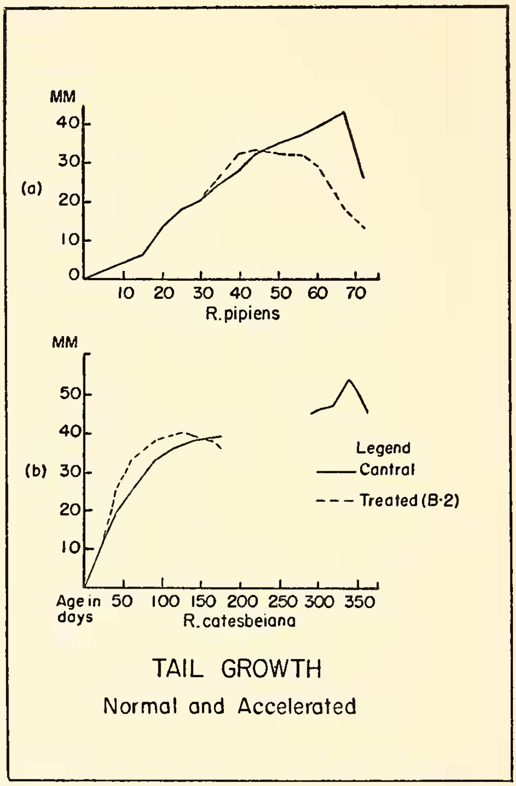
In the normal animal the pancreas progressively increases in size up to the time of metamorphosis when regression begins. The shape and the size of the pancreas were the same in treated and in untreated individuals of both species of the same age. Because regression cut short the normal growth period, the experimental animals' pancreas began regression at a smaller size than normal. Treatment with 1:10,000,000 thyroxine elicited a marked regression at the forelimb emergence stage and further shrinkage was observed in the terminal stage. There was no apparent regression in the pancreas of animals in the forelimb emergence stage which were treated with 1:100,000,000 thyroxine. At the onset of the terminal stage, regression was evident in both species.

c. *Gall Bladder*

Early in normal development the gall bladder was large as compared with the liver (about 1/5 its size). Later, it was relatively smaller (1/8 to 1/10 of the size of the liver). By the time of forelimb emergence, the gall bladder again appeared larger (1/5 to 1/4 the size of the liver). No measurements were made to show whether these size changes were changes in the gall bladder or in the liver, though it was apparent that the liver first increased and then decreased in size. Hoskins & Hoskins (1919 a & b) reported



Text-fig. 5. Relative hindlimb lengths in normal tadpoles and tadpoles treated with 1:100,000,000 thyroxine from 21 days of age. (a) *R. pipiens;* (b) *R. catesbeiana.* Age in days is plotted against the ratio of body length to hindlimb up to the tail stub stage in normal animals and to the "terminal" stage in the treated.



Text-fig. 6. Tail growth in normal tadpoles and tadpoles treated with 1:100,000,000 thyroxine from 21 days of age. (a) *R. pipiens;* (b) *R. catesbeiana.*

that the liver decreased in size during metamorphosis, and Kuntz (1922) reported an 80% reduction in liver weight which took place when tail resorption and intestine reduction were well advanced.

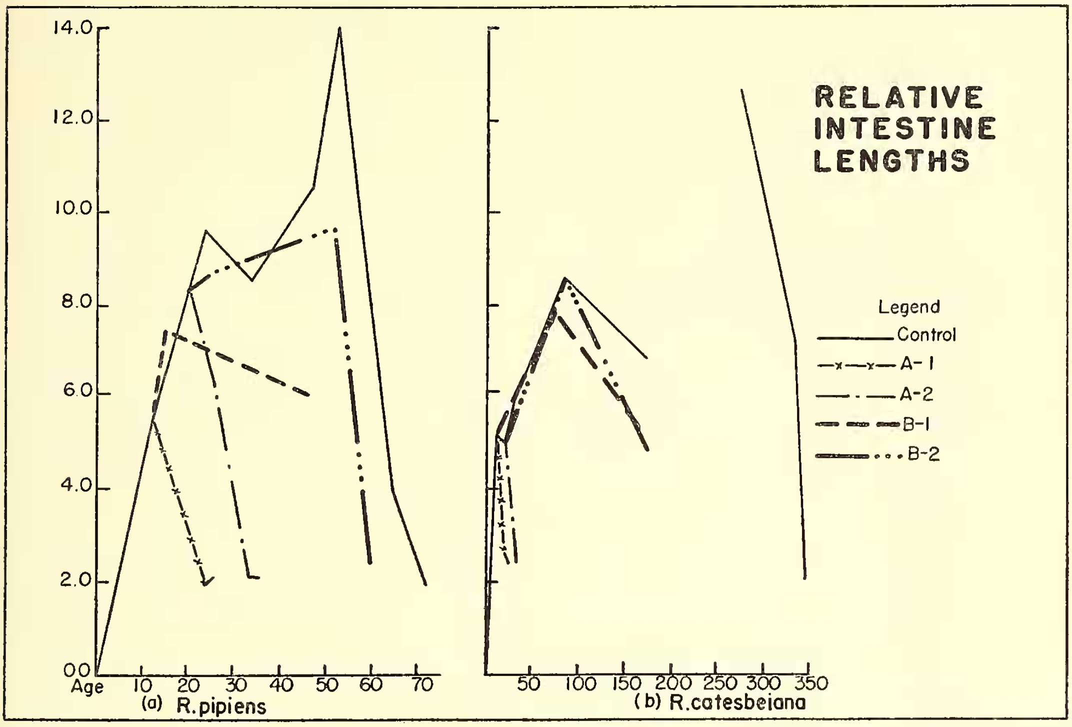
The gall bladders of animals treated with 1:10,000,000 thyroxine remained relatively large. At the terminal stage, they appeared relatively larger than in normal animals at the tail stub stage and the livers appeared to be more than normally reduced in size. The gall bladders of tadpoles treated with 1:100,000,000 thyroxine were still 1/8-1/10 of the size of the liver at the forelimb emergence stage.

Color change in the gall bladder during accelerated metamorphosis was studied in *Hyla versicolor, H. crucifer, R. clamitans, Bufo americanus* and *Ambystoma maculatum* (*A. punctatum* fide Speidel) by Speidel (1926). He noted no exceptions in several hundred observations from the series: "yellow-green," "brighter-green," "emerald green" and a "very dark green." In normal *R. pipiens,* the gall bladder was dark green at the time of forelimb emergence. In *R. catesbeiana* the gall bladder was emerald green at this time, and it was dark green at the tail stub stage. In most of the 1:10,000,000 thyroxine-treated *R. pipiens* tadpoles, the gall bladder was dark green at the forelimb emergence stage; in *R. catesbeiana,* at the terminal stage, the gall bladder was still emerald green as noted above. In the 1:100,000,000 thyroxine-treated tadpoles of both species, the gall bladder was emerald green at the forelimb emergence stage and approximately half of these individuals had dark green gall bladder at the terminal stage.

d. *Urinary Bladder*

The urinary bladder developed late in the normal premetamorphic climax period and was small but well developed in the tadpole with fully developed hindlimbs.

At the forelimb emergence stage of both species which had been treated with 1:10,000,000



TEXT-FIG. 7. Relative intestine lengths. Age in days is plotted against the ratio of intestine length to body length for *R. pipiens* in (a) and for *R. catesbeiana* in (b). A-1 curves are for tadpoles treated with 1:10,000,000 thyroxine from 12 days of age; A-2 for those treated with 1:10,000,000 thyroxine from 21 days of age; B-1 for those treated with 1:100,000,000 thyroxine from 12 days of age; B-2 for those treated with 1:100,000,000 thyroxine from 21 days of age.

thyroxine, approximately half had urinary bladders. At the terminal stage, all but one or two animals had small, poorly developed urinary bladders. In both species, the animals treated with 1:100,000,000 thyroxine had well-developed urinary bladders by the time of the forelimb emergence period.

e. *Tongue*

No measurements of the tongue were made for *R. pipiens*. In *R. catesbeiana*, normal development of the tongue began (when the tadpoles reached 21-27 mm. body length) as a white condensation of tissue in the anterior part of the floor of the mouth. At a body length of 35-38 mm., the tongue was clearly outlined and the tissue mass was about 1 mm. long. At the time of forelimb emergence, the tongue was a well-formed organ, 2.5-3 mm. long, with the posterior edge free and bifurcated. Between the end of forelimb emergence and the onset of the tail stub stage, the tongue grew rapidly and almost doubled its length to 5 mm.

All the experimental animals of both species showed more advanced tongue development than normal animals of the same age. In the groups treated with 1:10,000,000 which were examined at the terminal stage, the tongue was poorly differentiated, i.e., a free but not bifurcate posterior edge. In the groups treated with 1:100,000,000 thyroxine, the tongue appeared to be the same as in corresponding stages of normal animals. The amount of tongue growth (in length) between the forelimb emergence and the terminal stages for thyroxine-treated *R. catesbeiana* is compared with that in the normal in the table below. Also included is the time in days between the two stages. (The A-1 series, treated with 1:10,000,000 thyroxine from 12 days of age, is omitted because the tongue measured less than 0.5 mm in length at the forelimb emergence stage).

The slower growth rate of the tongue in organisms treated with 1:100,000,000 thyroxine is marked (See B-1 and B-2).

In the normal *R. pipiens* tadpole there are two premetamorphic tongue papillae; in *R. catesbeiana* there are four. These begin to be resorbed in the late tadpole stages and disappear by the onset of tail stub stage. In all the *R. pipiens* series, the papillae were gone by the beginning of the forelimb emergence, but in *R. catesbeiana*, Tongue Growth (in mm.)

Stage	*Control*	*A-2**	*B-1***	*B-2****
Forelimb Emergence	2.7	0.5	1.3	1.3
Tail Stub (Terminal)	5.0	0.7	2.2	1.8
% Increase	85%	40%	69%	38%
Days between Stages	7	2	82	90

* Treated from 21 days of age with 1:10,000,000 thyroxine.

** Treated from 12 days of age with 1:100,000,000 thyroxine.

***Treated from 21 days of age with 1:100,000,000 thyroxine.

papillae were still present at the terminal stage of animals treated with 1:100,000,000 thyroxine.

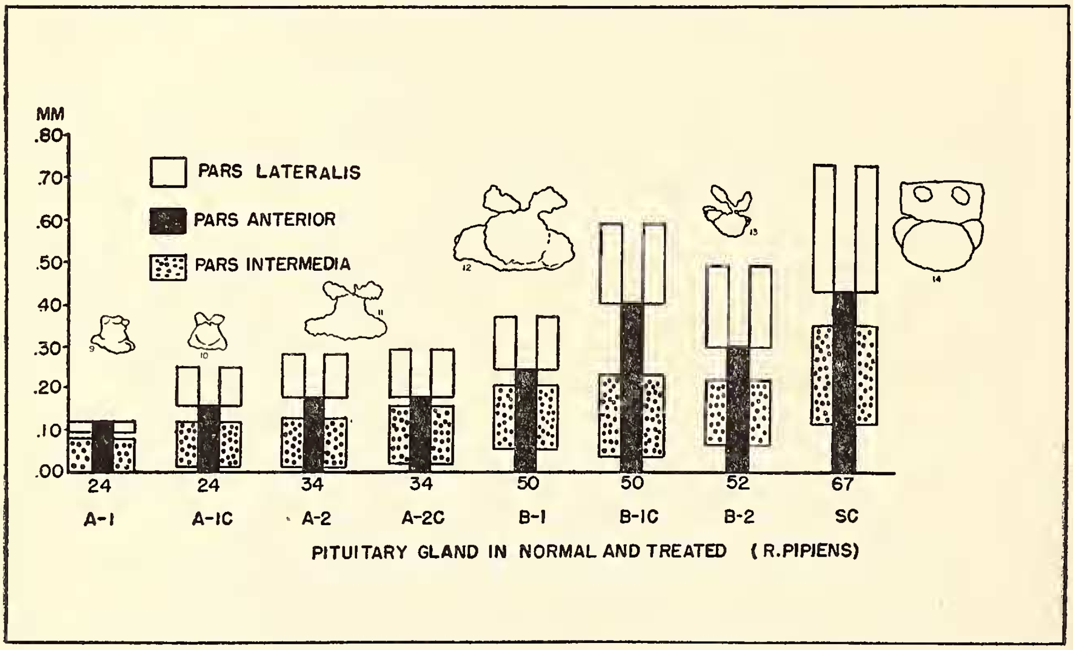
The histology of the premetamorphic tongue papillae of *R. sylvatica* was studied by Helff & Mellicker (1941) who found four papillae, the characteristic number for that species. In their search of the literature, they found that one previous investigator had described these structures. Schulze (1870, cited in Helff & Mellicker, 1941) found two papillae in *Pleobates fusca, R. esculenta, R. temporaria* and *Bufo cinereus. R. catesbeiana* thus resembles *R. sylvatica* in possessing four papillae, whereas *R. pipiens* resembles the species studied by Schulze.

3. *Histological Studies: Forelimb Stages, R. pipiens*

a. *Pituitary Gland*

i. *Anatomy*

In Text-fig. 8 are reproduced Atwell's (1918) reconstructions of the development of the anuran pituitary gland. It can be seen that two changes take place: first, the two lobes of the pars lateralis come to lie anterior and lateral to and (in the adult) separate from the pars anterior and pars intermedia; second, the mass of the gland grows posteriorly, the Anterior at a more rapid rate than the Intermedia (so that in the adult the former is the most posterior part of the gland. Also in Text-fig. 8, the length of each of the three components of the pituitaries of single normal and experimental animals is plotted in relationship to other parts. These dia-



Text-fig. 8. The pituitary in normal and treated *R. pipiens*. The drawings numbered 9 through 14 above the block graphs are redrawn from Atwell's (1918) reconstructions of pituitary development in Anura. The block graphs show the length and spatial relationships of the pituitary parts of normal and treated *R. pipiens* tadpoles obtained by counting the 10 micra sections each part occupied in each animal. A-1 is data from a forelimb stage animal 24 days old treated with 1:10,000,000 thyroxine from 12 days of age; A-1-C is from an untreated tadpole 24 days old; A-2 is from a forelimb stage animal 34 days old treated with 1:10,000,000 thyroxine from 21 days of age; A-2-C is from an untreated animal 34 days old; B-1 is from a forelimb stage animal 50 days old treated with 1:100,000,000 thyroxine from 12 days of age; B-1-C is from an untreated tadpole 50 days old; B-2 is from a forelimb stage animal 52 days old treated with 1:100,000,000 thyroxine from 21 days of age; SC is from an untreated forelimb stage animal 67 days old.

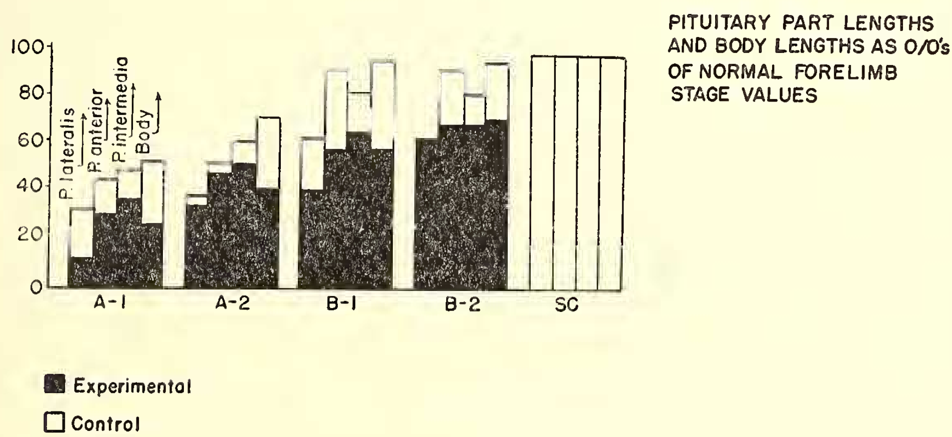
grams result from plotting the number of 10 micra sections constituting each part of the pituitary.

In the 12-day-old, 1:10,000,000 thyroxine-treated forelimb stage (A-1), pituitary relationships correspond to those in Atwell's youngest animal (stage 9), while the untreated animal of the same age (A-1-C) had progressed to Atwell's stage 10. In the 21-day-old, 1:10,000,000 thyroxine-treated forelimb stage animal (A-2), these appear to be the same as in the untreated animal of the same age. Both correspond to Atwell's stage 10 or 11. The higher concentration of thyroxine did not cause precocious anatomical development of the pituitary.

The pituitaries of the animals treated with 1:100,000,000 thyroxine from 12 days of age (B-1) and those treated from 21 days of age (B-2) were smaller than those of either the normal tadpole of the same age (B-1-C) or of the normal forelimb stage animal (SC). The block graphs do not show the progressive change in the connection between the two lobes of the pars lateralis with the pars anterior illustrated by Atwell's figures 12, 13 and 14. Examination of the slides showed that the normal tadpole (B-1-C), on the basis of lateralis position, had reached a stage between Atwell's 12 and 13 while the normal animal (SC) was between stages 13 and 14. The forelimb animals treated with 1:100,000,000 thyroxine (B-2 and B-4) appear to resemble the normal tadpole more than the normal forelimb emergence stage. The lower concentration of thyroxine, moreover, showed no accelerating effect on the anatomical development of the pituitary.

ii. *Relative size*

Text-fig. 8 also shows that the pituitaries of



TEXT-FIG. 9. Pituitary part lengths and body lengths of thyroxine-treated forelimb stages compared with those of the normal forelimb stages in *R. pipiens*. The length of each pituitary part and the body length of the experimental forelimb stages and of normal tadpoles of the same age as the treated are shown as percents of the normal forelimb stage values (SC, last set of four 100% blocks). In A-1, data from a forelimb stage animal treated with 1:10,000,000 thyroxine from 12 days of age are shown in black blocks superimposed upon that of an untreated animal of the same age in white. In A-2 the data from a forelimb stage animal treated with 1:10,000,000 thyroxine from 21 days of age are shown in the same way against the data from an untreated tadpole of the same age; B-1 are data from a forelimb stage animal treated from 12 days of age with 1:100,000,000 thyroxine shown against that for an untreated tadpole of the same age; B-2 data from a forelimb stage animal treated from 21 days of age with 1:100,000,000 thyroxine are shown against the data for an untreated tadpole of the same age.

all of the experimental forelimb-emergence animals were shorter than those of untreated animals of the same age. To determine whether the reduced size of the pituitary was proportional to the reduced body size of the thyroxine-treated animals and to determine whether or not all pituitary parts were equally affected, the length of each pituitary part and the body length of the normal forelimb stage animal were taken as 100%. The same measurements for each experimental forelimb animal and for its age control were recalculated as percents of the lengths of the corresponding parts in the normal forelimb animal. In Text-fig. 9, the profiles produced by graphing these values for the experimental animals were superimposed in black upon the profiles of their respective age controls. The last profile to the right (a block of four 100% columns) represents the values for the normal forelimb stage animal.

It can be seen that the bodies of those treated with 1:10,000,000 thyroxine and those treated with 1:100,000,000 thyroxine appeared to have bodies and pituitaries about equally inhibited (with regard to length) at the forelimb stage, and in these animals the pars anterior appeared to be the pituitary component most inhibited.

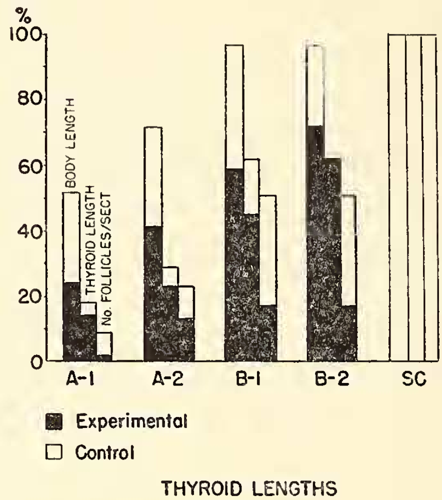
iii. *Cellular Differentiation*

Distribution and differential counts of the pituitary cell types were not considered because of the thickness of the sections. The cytoplasm in the cells of all the lobes was less abundant in the experimental animals than in either the age controls or the normal forelimb stage. Pigment granules which were large and coarse in young normal stages were more evident in all the experimentals than in the stage controls.

b. *Thyroid Gland*

i. *Size of the Gland*

Conclusions as to thyroid size were based on the length of the glands determined by counting the number of 1 micra sections which the two halves of the gland occupied in each animal, and on a relative area per section derived from counting the number of follicles containing visible colloid. To show how the thyroids of the thyroxine-treated forelimb stage animals differed from those of the normal forelimb stage animal and from the untreated tadpoles of the same age, body length, length of thyroid and average number of colloid-filled follicles per section in the normal forelimb stage animal were arbitrarily rated as 100%. The same data for the treated animals (forelimb stage) and for their respective age controls were recalculated as percents of these norms. In Text-fig. 10 are five profiles resulting from this treatment of the data. At the extreme right is the normal forelimb stage profile. To the left are the profiles for the untreated tadpoles. Superimposed upon these are the profiles of the treated forelimb stage animals of the same age.



TEXT-FIG. 10. Thyroid size and body size in normal and thyroxine-treated *R. pipiens.* Body length, length of thyroid determined by counting the number of 10 micra sections the gland occupied and the average number of follicles per section for the accelerated forelimb stages and for untreated animals of the same ages as the treated are shown as percents of the normal forelimb stage values (SC, the last set of three 100% blocks). The values for each treated forelimb stage animal are shown in black blocks superimposed on the same values for a normal tadpole of the same age shown by white blocks. A-1 shows the values for a forelimb stage animal treated from 12 days of age with 1:10,000,000 thyroxine and for a normal animal of the same age; A-2, for a forelimb stage animal treated from 21 days of age with 1:10,000,000 thyroxine and for a normal tadpole of the same age; B-1 for a forelimb stage animal treated from 12 days of age with 1:100,000,000 thyroxine and for a normal tadpole of the same age; B-2 for a treated forelimb stage animal treated from 21 days of age with 1:100,000,000 thyroxine and for a normal tadpole of the same age.

Text-fig. 10 shows that the thyroids of the treated animals at the forelimb emergence stage were inhibited as reflected by their size when compared with the normal forelimb stage and untreated animals of the same age. Inhibition as indicated by area (determined by the number of follicles) was greater than that indicated by length.

ii. *Mitotic Activity*

In the following table are given the number of 10 micra sections occupied by both the right and left halves of the thyroid glands, and the number of mitoses in the entire glands.

	Sections	*Mitoses*
A-1	30	3
A-1-C	39	47
A-2	49	12
A-2-C	62	120
B-1	95	14
B-1-C	130	406
B-2	131	24
SC	211	861

The normal series—A-1-C, A-2-C, B-1-C and SC—gave evidence of higher mitotic activity than did the treated animals.

iii. *Glandular Activity*

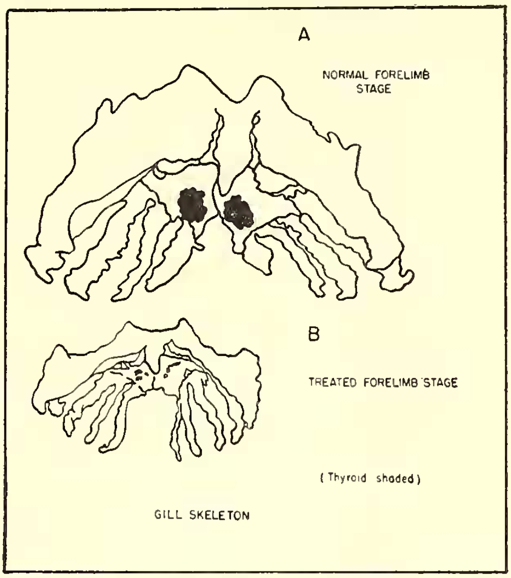
A thyroid gland made up of vacuolate columnar cells, with vacuolate colloid, and of many collapsed follicles, possesses the generally accepted histological criteria of a physiologically active gland. The forelimb stage animals treated from 12 days of age with 1:10,000,000 thyroxine (A-1) had thyroid glands made up of cuboidal cells with scant cytoplasm, with few follicles, and with no vacuolation. In those treated with 1:100,000,000 thyroxine from 21 days of age (A-2), the thyroid cells were cuboidal. The cells had more cytoplasm than those of the A-1 animals but less than those in animals of the same age. As in the age controls, a few follicles showed vacuolation of the colloid. The animals from both age groups treated with 1:100,000,000 thyroxine had thyroids in which the cuboidal cells were approaching a columnar architecture. The thyroids of the normal forelimb stage animals bore all of the criteria of high activity.

iv. *Pigment*

In the younger normal animals (as was noted by D'Angelo & Charipper, 1939) large aggregates of pigment are conspicuous features of the thyroid gland. By the time of forelimb emergence, however, such aggregates were rare. Moreover, the granules are distributed and the pigmentation is not conspicuous. All of the experimental animals showed pigmentation either to the same degree or to a greater degree than their respective age controls.

v. *Thyroid Position*

Text-fig. 11 shows outline drawings from graphic reconstruction of the thyroid gland and its relationship to the ventral aspect of the hyobranchial cartilages. Text-fig. 11a shows the normal forelimb emergence stage (5×). Text-fig. 11b is the forelimb emergence stage after 1:100,000,000 thyroxine treatment from 12 days of age (enlarged 11.25×). The thyroid gland assumed a more anterior position under the gill-bearing skeleton in the treated animals than in the normal. D'Angelo & Charipper's (1939) Figure 1, illustrating the normal changes in thyroid position up to and including metamorphosis, indicates that the more anterior position is characteristic of normal tadpoles of the same age as the treated. The position of the thyroid in the normal forelimb stage agrees with their observations from the same stage. Thus 1:10,000,000 thyroxine treatment did not effect a precocious migration of the thyroid.



TEXT-FIG. 11. Thyroid position relative to the gill skeleton in the normal forelimb stage and in the forelimb stage produced by treatment with 1:100,000,000 thyroxine from 12 days of age. Graphic reconstruction from serial sections. Normal stage magnified 5×; treated 11.25×. The thyroid is shaded.

c. *Small Intestine*

In the normal forelimb stage animal, the histology of the small intestine is well-represented by Kuntz's (1922) figures for the same stage of *R. pipiens* and by Jane's (1934) illustration for this stage in thyroid-fed tadpoles of *R. clamitans*. There were many mitoses in the basal cells of the new mucosal layer. The old mucosa was evidenced by conspicuous necrotic masses. The submucosa and muscularis had thickened considerably. The forelimb stage animals treated with 1:10,000,000 thyroxine had the intestinal histology of the normal forelimb stage. However, the cytoplasm of the cells in all layers was very scant and the resulting histology was superficially quite different from the normal. Forelimb stage animals treated with 1:100,000,000 thyroxine had the same intestinal histology as that of the untreated tadpoles of the same age.

d. *Pancreas*

In the normal forelimb stage animal regression of the pancreas was far advanced. The histological picture represented a condition intermediate to Kaywin's (1936) stages 3 and 4 of thyroxine-treated *R. catesbeiana.* The acini showed three stages of metamorphic changes: (1) some were necrotic, (2) some were made up of syncytial cells, and (3) some were made up of cells with large nuclei and sparse, deeply-staining cytoplasm. There were pronounced connective tissue spaces.

In the forelimb stage of animals treated with 1:10,000,000 thyroxine, the pancreas was comparable to the normal forelimb stage pancreas as regards the degree of metamorphic change. However, pigment was very conspicuous. Forelimb stage animals treated with 1:100,000,000 thyroxine failed to show metamorphic change in the pancreas. The glands were smaller than those of the untreated tadpoles of the same age, but there were no signs of loss of definition of cell boundaries or necrosis in the acinar cells.

e. *Summary of Observations on the Histology of the Forelimb Stages of R. pipiens*

Kahn (1916) found that the pituitaries of tadpoles treated with horse thyroid were much larger than normal. Schliefer (1935) found that thyroid extract had no effect on the pituitary although the development of the gland was accelerated along with the rest of the body. Under the conditions of the present study the anatomical development, at the forelimb emergence stage, of the pituitary of thyroxine-treated animals was not found to be more advanced than that of untreated animals of the same age. In fact, the glands were smaller. Treatment with 1:10,000,000 thyroxine was less inhibitory to pituitary growth than it was to body growth. Thus, in agreement with Kahn, the pituitaries were larger than those of untreated tadpoles of the same age. After treatment with 1:100,000,000, body size and pituitary size were in a more normal proportion; with this concentration, the pars anterior appeared to be more inhibited than the other pituitary components. With both concentrations, the cytoplasm of the cells of all parts of the pituitary gland was sparse and pigment remained conspicuous in the pars anterior and pars lateralis.

The thyroids of treated animals showed evidence of inhibition in every respect considered at forelimb emergence. They were much smaller, had a lower mitotic rate and give histological evidence of a lower degree of differentiation and physiological activity than the glands of either the normal forelimb stage or of the untreated tadpoles of the same age. These findings agree with those of Clements (1932), Etkin (1935) and Brinks (1936).

The small intestine and pancreas in animals treated with 1:10,000,000 thyroxine showed the same type of histological change at the time of forelimb emergence as that of normal animals at the same stage. On the other hand, the intestines and pancreases of those treated with 1:100,000,000 thyroxine did not show metamorphic change.

4. *General Summary of Observations*

A comparison of the condition of seven internal organs, in normal metamorphic stages and in comparable stages produced by treatment with two concentrations of thyroxine, has shown that at each stage, the accelerated animals are different not only from the normal but also from each other. This is summarized, in the table below, for the forelimb stage.

Difference in the length of the tadpole period in *R. pipiens* and in *R. catesbeiana* did not greatly affect the results of thyroxine treatment as studied by growth measurements and dissection. *R. catesbeiana* reacted earlier, considering its normal tadpole period, to both concentrations of thyroxine than did *R. pipiens.* In days, *R. catesbeiana* reacted more quickly to 1:10,000,000 thyroxine and less quickly to 1:100,000,000 thyroxine than *R. pipiens.* As judged from the degree of response, *R. catesbeiana* was less sensitive than was *R. pipiens,* as shown by the fact that resorption of the tail and shortening of the intestine after 1:100,000,000 thyroxine treatment was not as advanced. In comparison with their respective normal stages, nevertheless, the animals showing accelerated development behaved similarly in both species.

Discussion

The thyroid glands themselves of animals undergoing thyroxine-accelerated metamorphosis apparently do not modify the effects of thyroxine treatment. Etkin (1935) reached this conclusion by comparing the effects of thyroxine on thyroidectomized and partially thyroidectomized *R. cantabrigensis* tadpoles. The present study supports that conclusion by the detailed similarity of the external metamorphosis of acceler-

NORMAL DEVELOPMENT IN NORMAL AND TREATED FORELIMB STAGE ANIMALS

	Normal	Thyroxine-treated (1) 1:10,000,000	Thyroxine-treated (2) 1:100,000,000	Age Controls for (1) and (2)
1. Intestine				
a. Shortening	¾–*R. pipiens* ½–*R. catesbeiana*	Complete	Little or none	None
b. Histology (*R. pipiens*)	Tadpole & adult	Tadpole & adult	Tadpole	Tadpole
2. Pancreas				
a. Regression	Evident	Excessive	Not evident	None
b. Histology (*R. pipiens*)	Tadpole & adult	Tadpole & adult	Tadpole	Tadpole
3. Gall Bladder				
a. Relative size (relation to liver)	⅕–¼	¼–⅓	⅛–1/10	⅛–1/10
b. Color	Emerald (*R. catesbeiana*) Dk. green (*R. pipiens*)	Emerald Dk. green	Emerald	(1) Yellow-green (2) Pale green
4. Urinary Bladder	Well-formed	None in ½; poorly developed in ½	Well-formed	None
5. Tongue				Tissue condensation in (1); well-formed in (2)
a. Shape	Well-formed	Immature	Well-formed	
b. Premetamorphic Papillae	Regressive	Gone (*R. pipiens*) Regressive (*R. catesbeiana*)	Gone Regressing	2 present in *R. pipiens* 4 present in R. *catesbeiana*
6. Pituitary (*R. pipiens*)				
a. Anatomy	Atwell's stage 13-14	Atwell's stages 9 and 10	Atwell's stage 12 or 13	Stage 10 and 11 for (1) Stage 12 or 13 for (2)
b. Size				
i. Relative to age norm		Smaller	Smaller (especially pars anterior)	
ii. Relative to body size		Larger than normal	About normal	
7. Thyroid (*R. pipiens*)				
a. Size	Large	Very small	Small	Increasing
b. Histology	Climax	Retarded	Retarded	
c. Mitoses	Many	Few	Few	Many
d. Position	Migrating	Not migrating	–	–

ated intact *R. pipiens* and *R. catesbeiana* to that of his accelerated, thyroidectomized animals. The development of the thyroids of treated *R. pipiens* at the time of forelimb emergence was very much inhibited and resembled that of hypophysectomized tadpoles described by Atwell (1935) seventeen months after operation. The growth of the pituitaries of these animals was also inhibited. D'Angelo (1941) correlated the growth and differentiation of the thyroid with the marked growth and basophilic differentiation of the pituitary pars anterior during the period of rapid hindlimb growth. Inhibited pituitary development is a plausible explanation for the inhibited condition and apparent inactivity of the thyroids of thyroxine-treated tadpoles.

In attempting to duplicate the normal pattern of sequence and spacing of the external metamorphic events, Etkin (1935) found that no one concentration of thyroxine could effect this result. However, by starting with low concentrations and gradually increasing the dosage, he obviated both the simultaneous occurrence of events normally spaced in time which resulted from the use of single high concentrations and the greater than normal intervals between events which resulted from the use of single low concentrations. Schreiber (1934 a & b) and Gache (1940) account for both the sequence and the spacing of events in normal metamorphosis as results of differences in the response thresholds of the several organs at the level of thyroxine increases. Etkin, having found the sequence of events unchanged by different concentrations of thyroxine, considered that this sequence was inherent in the tissues, all of which were sensitive even to the lowest concentrations, and concluded that only the spacing of events and rate of response were thyroxine controlled.

If the sequence of events is controlled by increasing concentrations of hormone reaching successively the low thresholds of early events and the higher thresholds of later events, then a particular concentration capable of initiating, let us say, a late event must also be able to initiate other events normally occurring earlier or at the same time. If the sequence of events is inherent in the tissues and is independent of the concentration of thyroxine, then if a particular event has already taken place in both normal and accelerated animals, other events already begun in normal animals should also have begun in the accelerated animals regardless of the concentrations used.

In the present experiments, normally metamorphosing and accelerated animals were examined at a time when, in each, the same external metamorphic event had just occurred, namely, forelimb emergence. In the normal animal, the histology of the intestine and pancreas is midway in the change to the adult condition. In animals treated with 1:100,000,000 thyroxine, there was no histological change apparent in either organ, while those treated with 1:10,000,000 thyroxine resembled the normal in histology. The urinary bladder is absent in half the forelimb stage animals treated with 1:10,000,000 thyroxine, but it is present in the normal forelimb stage, the normal late tadpole, and in the 1:100,000,000 thyroxine-treated animals in the forelimb stage. The gall bladders of 1:100,000,000 thyroxine-treated animals retain the tadpole size relation to the liver, but in the normal forelimb stage and in the 1:10,000,000 thyroxine-treated forelimb stage, the gall bladders are increased in size relative to the liver.

If the delayed events cited were just begun although not far advanced, then these deviations in the accelerated animals from the normal integration of events could be explained as concentration effects upon the reaction rates and, thus, upon the spacing of events, rather than as an alteration of sequence. This would corroborate Etkin's views. Despite the difficulty imposed by the conditions of the present experiments in ascertaining precisely the initiation of an internal metamorphic event, the probability of its having occurred in normal sequence regardless of the thyroxine concentration can be further explored if its position as one of a sequence of three events is followed.

In normal animals, the urinary bladder (1) is well formed and intestinal shortening (2) is ½ to ⅔ complete when the forelimbs emerge (3). In 1:100,000,000 thyroxine-treated animals, the urinary bladder (1) is well formed at forelimb emergence (3) but there has been little or no shortening of the intestine (2). In 1:10,000,000 thyroxine-treated tadpoles, all have fully shortened intestines (2) at forelimb emergence (3) but half have no urinary bladders (1). At any given moment in normal metamorphosis, the rising concentration of thyroxine is the same for all organs and although, as Etkin suggests, each event may have its own rate to be speeded or retarded by concentration, this rate at normal concentrations is necessarily related to those of other events taking place at the same time. If all the events were initiated in the natural sequence in the development of the animals treated with the two concentrations, the cited deviations in developmental pattern from the normal in the two groups can be ascribed entirely to the effects of the experimental concentrations on rate. It seems improbable that the urinary bladder, normally formed before intestinal shortening begins, should not be visible under 17× magnification in half the 1:10,000,000 thyroxine-treated animals at the forelimb stage, all with fully shortened intestines, unless the beginning of its development had been delayed relative to the natural or normal developmental sequence.

If the normal sequence of events is not a matter of successive threshold responses (since a concentration that produced one event did not produce all the other naturally concurrent events) and is not independent of thyroxine contration (since both concentrations apparently produced altered sequence), another explanation for the control of metamorphic sequence must be sought.

The lack of significant differences in response to the same thyroxine concentrations in the two species studied indicates that differences in tissue sensitivity are not the cause of the species differences in the length of time before the onset of metamorphosis. This, taken with the precocious thyroid activity and metamorphosis produced by Ingram (1928) in *R. catesbeiana* with *R. clamata* pituitary implants; with Etkin's (1950) demonstration of tissue sensitivity to thyroxine as early as the opercular closure stage; and with the well-known effects of hypophysectomy on metamorphosis, leads to the conclusion that the onset of metamorphic change is pituitary-controlled. It has been accepted that this control is exerted through trophic action on the thyroid gland. If the sequence of metamorphic events is neither solely an effect of the thyroid in producing rising thyroxine concentrations nor independent of concentration and inherent in the tissues, perhaps the control of metamorphic sequence may be found in a thyroid-pituitary interrelationship at the tissue level.

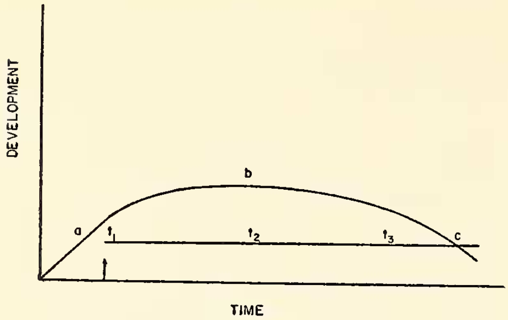
It is evident that a dynamic equilibrium must be maintained between growth and differentiation during the late tadpole and early metamorphic period. Tadpole organs continue to grow up to the time the changes take place which cause them either to disappear or differentiate, producing the adult condition. Potential adult organs both grow and differentiate during the tadpole period. That both the thyroid and pituitary are involved has been shown in the results of hypophysectomy and thyroidectomy. Smith (1916 a & b, 1918) showed that although the initial growth rates of normal and hypophysectomized tadpoles were very similar, a definite retardation of growth became evident in the second half of the tadpole period during which growth is normally rapid. Thus, the effect of the pituitary upon growth begins to be exerted at the period of rapid thyroid, hindlimb and general body growth. Hoskins & Hoskins (1919 a & b) found that thyroidectomized tadpoles grew more rapidly and were almost twice the size of normal animals at the time they should have metamorphosed. Gordon, Goldsmith, & Charipper (1943, 1945) also reported excessive growth in chemically thyroidectomized tadpoles. These results suggest that, after an initial independent period, growth during the second part of the tadpole period is affected by two factors: (1) a factor from the pituitary-promoting growth and (2) a factor from the thyroid-inhibiting growth. That the latter normally overtakes the former may be inferred from the cessation of growth at metamorphosis when thyroid activity is at its peak. It is of interest to note here that in accelerated metamorphosis, in an organ like the pancreas which normally develops a typical structure that is replaced by a different adult structure at metamorphosis, the acceleration does not consist of a telescoping in time of the normal anatomical history of the organ. Instead, the tadpole part is dropped out. The initiation of metamorphic sequence may depend upon a succession of tissue-determined critical ratios between the concentrations of the pituitary growth factor and thyroxine.

In an early period, the initiation of development in adult organs might take place in an order determined by the concentration of thyroxine and the growth stimulus, while tadpole organs unaffected by the low thyroxine inhibition continue to grow; in an intermediate period, growth of all organs continues followed by a later period in which growth slows in all organs and as the growth/thyroxine ratio changes, successive tadpole organs begin metamorphosis.

This explanation, based on the growth curves shown in Text-fig. 2 and applied to the conditions under 1:10,000,000 thyroxine treatment, might be present as follows.

Because the experiments began in the period which Smith found independent of hypophysectomy, the cessation of growth observed would indicate that pituitary independent growth is also inhibited by thyroxine in this concentration. Tadpole growth rapidly ceased, regressive changes were marked, and adult organs appeared but were small and underdeveloped. The pituitary and thyroid were retarded compared with the glands of untreated tadpoles of the same age.

In the 1:100,000,000 thyroxine experiments, tadpole growth continued. At first, this was a virtually normal rate, but as time went on the growth curves flattened. The conditions in 1:100,000,000 thyroxine acceleration might be represented as follows (Text-fig. 12). Here "a" is the period of pituitary independent growth,



TEXT-FIG. 12. For explanation see text below.

"b" is growth affected by 1:100,000,000 thyroxine ("c") applied from the time indicated by the arrow. Three different periods in the relationship of "a"—"b" to "c" can be recognized, but each differs from the corresponding period in the "normal" diagram. In period t_1, 1:100,000,000 thyroxine is applied; the growth rate increases whereas the level of thyroxine does not. The thyroxine concentration is too low to stop tadpole growth, but is sufficient to initiate the growth of the adult organs.

In period t_2, the relation of "b" and "c" is fairly constant, as can be seen (Text-fig. 12). In period t_3, "b" decreases slowly and "c" remains constant. As a result "b" approaches "c" more slowly and the period t_3 is longer. This lateration in the amount of change and the timing of the change in the growth/thyroxine relationship could account for the relatively longer limbs of the 1:100,000,000 thyroxine-treated animals, the slower-than-normal tail resorption and the delay in intestinal shortening. That tail resorption eventually takes place in some specimens, as does intestinal shortening, can be explained by the growth factor falling slowly in value to a point where, although the thyroxine concentration has not been changed, the normal "critical" ratio between the two factors is approached. This would also bring Allen's (1932) statement that "an apparently subminimal concentration of thyroxine may be effective if it acts long enough" into conformity with subsequent evidence that thyroxine is not stored in the tissues (Etkin, 1935).

Experiments on starved tadpoles have produced results of great interest in this connection. D'Angelo, Gordon & Charipper (1938) showed that starvation imposed before the 5-8 mm. hindlimb stage in *R. sylvatica* retarded development and resulted in failure of metamorphosis, but starvation imposed after this resulted in precocious metamorphosis. Later it was found (1941) that early starvation resulted in extreme retardation of the thyroid gland and failure of cell differentiation in the pituitary. Tadpoles starved at later stages had thyroids which showed secretory activity for some time. It was suggested that early metamorphosis may be the result of precocious thyroid activity possible from the increased sensitivity to thyrotrophic hormone. There is also a possibility that once the thyroid secretes hormone, a reduction in the growth potential by starvation could precipitate metamorphosis at a level of thyroxine production which would be insufficient normally. It may be necessary to point out that the smaller body size of the 1:100,000,000 thyroxine-treated animals in the present experiments is not the result of starvation from precocious cessation of feeding. The intestines were distended with food when examined histologically at the forelimb stage and feces were abundantly present in the thyroxine solutions at the daily changes until well after this period.

The explanation above has been offered to show how the normal sequence of metamorphic events might be controlled by a series of critical ratios between the growth-promoting influence of the pituitary and the effects of the thyroid. The experimental data offer little evidence concerning the function of the pituitary-accelerated metamorphosis. The pituitary glands were smaller than normal, on an absolute basis, in the treated animals but not relatively, as compared with body size. The pars anterior seemed to be more reduced than the other parts. The anatomy was tadpole rather than normal at the forelimb stage. The appearance of the thyroid indicated deficiency of thyrotrophic hormone. On such evidence elaboration of theory is premature and must await further data such as might be afforded, for example, by a comparison of the effects of thyroxine treatment on metamorphic sequence in intact and in hypophysectomized tadpoles.

SUMMARY

1. Comparison of the development of the intestine, pancreas, urinary bladder, tongue, gall bladder, pituitary and thyroid glands in normal and thyroxine-accelerated *R. pipiens* and *R. catesbeiana* tadpoles at similar metamorphic stages has shown that the accelerated animals differ from the normal and that those accelerated by 1:10,000,000 thyroxine were different from those accelerated by 1:100,000,000.

2. In the same animals, different organs showed either the same or more advanced development than did the corresponding organs of normal animals.

3. Treatment of *R. pipiens* tadpoles with either concentration resulted in less developed thyroids and pituitaries than were found in

naturally metamorphosed animals of the same stage.

4. The responses of the tadpoles of the two species to the same experimental treatment were much alike in every point compared despite the difference in the length of their tadpole periods.

5. The data fail to support the theory that the sequence of metamorphic events is dependent upon a series of thyroxine thresholds in the several organs, but indicate some probability that the sequence is dependent in part on thyroxine concentration.

6. It is suggested that normal metamorphic sequence may result from a thyroid-pituitary relationship which changes with time and operates at the tissue-cellular level rather than at the organ level.

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